Middle East Journal of Applied Science & Technology
Vol.5, Iss.4, Pages 13-17, Oct-Dec 2022

A Systematic Review on Isolation, Identification, and Characterization of *Agrobacterium tumefaciens* from Leguminous Plants

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DOI: https://doi.org/10.46431/MEJAST.2022.5403

Article Received: 29 August 2022 *Article Accepted: 24 October 2022* *Article Published: 10 December 2022*

ABSTRACT

Agrobacterium tumefaciens causes crown gall disease in plants by transferring their T-DNA which contains some oncogenic genes. At the site of the wound, these bacteria become accumulates and then transfer their T-DNA into host plant cells, which interact with host plant genomic DNA and cause tumor. With the help of knowledge, how the *A.tumefaciens* transfer their T-DNA to plant cells. And with the knowledge and use of the latest molecular biology techniques, *A.tumefaciens is* used as a tool for the transformation of our gene of interest into the host plant cells to make genetically modified plants. This review provides a working systematic review of the isolation, identification, and characterization of *A.tumefaciens* from the leguminous plants. This review also tells us how to transfer the T-DNA into a host plant cell and how to isolate the *Agrobacterium tumefacien*s from the root nodules of leguminous plants, first of all, collect the root nodules of leguminous plants then wash them properly and streak the isolates of root nodules on LB agar plates. then identify and characterize the *Agrobacterium* from the isolates because *Agrobacterium* and rhizobium stay together. For characterization, biochemical tests i.e gram staining and KOH tests performed to confirm the isolated bacteria is gram-negative. Some pathogenic tests such as carrot disk bioassay and potato disc confirm whether isolates caused a tumor or not.

Keywords: *Agrobacterium tumefaciens, T-DNA, KOH tests, Antibiotic-resistant tests, Gram staining, Genetically modified plants.*

1. INTRODUCTION

Agrobacterium tumefaciens is a soil-born, gram-negative, rod-shaped, motile bacteria having the peritrichous type of flagella that causes crown gall disease in plants. *A. tumefaciens* causes crown gall disease by entering through the site of the wound (Rahman *et al.,* 2020). *A. tumefaciens* is a soil-born pathogenic bacterium that belongs to the family of *Rhizobiaceae.* These are rod-shaped and gram-negative bacteria and grow aerobically. *A. tumefaciens* causes crown gall disease in plants by transferring their T-DNA which contains some oncogenic genes. At the site of the wound, these bacteria become accumulates then they transfer their T-DNA into host plant cells, which interact with host plant genomic DNA and cause tumor.

With the help of knowledge, how the *A.tumefaciens* transfer their T-DNA to plant cells. And with the knowledge and use of the latest molecular biology techniques, *A.tumefaciens is* used as a tool for the transformation of our gene of interest into the host plant cells to make genetically modified plants. Any gene of interest can replace the oncogenic part of the T-DNA region for the transformation (Hwang *et al.,* 2017).

The *Agrobacterium* genus contains bacterial species that live in soil microflora, where they live in the rhizosphere. However, species of *Agrobacterium* cause four types of diseases in various plants including the ‘‘crown gall tumor” which is caused by the *Agrobacterium tumefaciens*, “cane gall” caused by *Agrobacterium rubi*, and “hairy root” caused by *A. rhizogenes* and lastly *A. vitis* a new species of *Agrobacterium* cause the tumors and necrotic lesions on various types of plants, especially on the grapevine.

The virulent strains of *Agrobacterium* can infect several hundred to several thousand plants, these plants can be herbaceous plants or woody plants. Among these four types of *Agrobacterium* most important for studies is

Agrobacterium tumefaciens. Because *A. tumefaciens* can transmit their T-DNA to host plant cells to cause crown gall disease, this unique mechanism makes the bacterium more important for making transgenic plants, and this bacterium is used as a tool in plant breeding (Păcurar *et al.,* 2011).

The scientist collects some root nodules from healthy and young plants of *Sesbania aculeata* from different fields and different locations. Then isolate the isolates from the samples of the nodules of *Sesbania aculeata* and characterize the isolates with various kinds of tests including the ketolactose test, Hofer's test, and acid-alkaline production test. When the isolates are cultured on yeast extract mannitol agar (YEMA) containing congo red, all strains are gram-negative and did not absorb the color. The yellowish zone of Cu_2O is not found in the ketolactose test. In Hofer's test, no growth of isolates was shown which is the nature of rhizobia (Riah *et al.,* 2014).

Isolate the *A. tumefaciens* from the infected plants of peach (*Prunus perisca),* collect the crown gall samples from the infected plants of peach, and identify *A. tumefaciens*. They use MacConkey selection media for the identification of *A. tumefaciens,* this media is specifically used for *A. tumefacient* growth. KOH and gram staining are two biochemical tests they performed to confirm the isolated bacteria is gram-negative. They also perform pathogenicity tests such as carrot disk bioassay and potato disc to confirm whether isolates cause a tumor or not. They also perform antibiotic-resistant tests to check the resistance of isolates to rifampicin antibiotics. All the tests that they performed showed that the isolated bacteria were *A. tumefaciens* (Ali *et al.,* 2016).

In this experiment, the scientist use various leguminous plants including the chickpea (*Cicer arietinum)* Hepper (*Vigna mungo*), and mung (*Vigna radiata*) were isolated to identify and characterize *Rhizobium* strains. Biochemical and antibiotic sensitivity tests were also done. They collect 37 total samples from which 27 were *Cicer arietinum,* 5 samples of *Vigna radiata,* and 5 samples are *Vigna mungo* from different areas in India. The growth of the isolates on the bases of white mucoid on the yeast extract mannitol agar (YEMA) medium 18 samples from the *Cicer arietinum* and 3 samples from the *Vigna mungo* and no sample from *Vigna radiata* were found which contain *Rhizobium.* All 21 (18 from *Cicer arietinum* and 3 from the *Vigna mungo*) were found gram-negative and round-shaped in gram-staining reactions (Tyagi *et al.,* 2017).

Cicer arietinum (Chickpea) is a most important crop. A root-nodulating bacterium *Mesorhizobium cicero* shows symbiotic nitrogen fixation (SNF). These bacteria interact with other endophytic bacteria, but these bacteria cannot harm the plant. Endophytic microorganisms mainly belong to the *Mesorhizobium* for their operational role and development of chickpeas (Koli & Swarnalakshmi, 2017).

This experiment explores the characterization of *Agrobacterium*. Characterization of isolates is done by gram staining and biochemical tests of *Agrobacterium.* Polymerase chain reaction (PCR) is also used to characterize isolates using different primer sets for the amplification of some specific regions of ribonucleic acid (DNA) of the *Agrobacterium.* For the identification of the isolate's family, use the AGRH set of primers for the identification of *Rizhobieaceae. BIOVAR1* set of primers used to identify the *Agrobacterium BIOVAR* group 1. *VIRG* third set of primers used to identify the presence of Virg, Virg region is only present in pathogenic strains of *Agrobacterium* (Finer *et al.,* 2016).

In this experiment, the scientist performs various kinds of tests for the characterization of *Agrobacterium* from isolates of various leguminous plant nodules. The *Agrobacterium* characterization was based on physical,

biochemical, and cultural characteristics which include the 3-ketolactose test, Congo red test, potato dextrose test (PDA) test, glucose peptone agar (GPA) test, and pathogenicity tests (Murugesan *et al.*, 2010).

2. MATERIALS AND METHODS

2.1. Isolation

The healthy and unharmed plant samples of chickpeas (*Cicer arietinum)* were collected from different fields. The plants were uprooted and shaken to remove the loosely attached soil, and then the nodules were washed with water to remove the adhered soil. The nodules were separated carefully and sterile according to the procedure of Mir *et al.* (2021). Select the larger-size nodules to isolate the bacteria. Wash the nodule with 90% alcohol for 5-10 seconds. Then nodules were submerged in 0.1% mercuric chloride solution for 5 minutes (Murugesan *et al.* (2010b). Then rinse with sterile distilled water thoroughly six times to remove the chemicals. Then crush the nodules aseptically and streak them on Agar plates and incubate these plates at 28 degrees Celsius for 3-4 days. The growth of rhizobium will be shown on plates, then isolate the *Agrobacterium tumefaciens* by using selective media. The bacteria will grow on the plates and after incubation develops into colonies. Growth will be maintained according to the procedure of Koli & Swarnalakshmi Koli & Swarnalakshmi, (2017) single colony of isolates grows on a nutrient agar medium, and the working cultures are saved in agar plates at 4 degrees Celsius.

2.2. Identification and Characterization

Some tests were performed for the identification and characterization of *A. tumefaciens* from the isolates of nodules of plants.

2.3. Antibiotic resistance tests of isolates

Different antibiotics like cefotaxime, rifampicin, kanamycin, and tetracycline were used to check the resistance of the bacterium to antibiotics. A 20-microliter bacterial culture was used for the antibiotic test. And 10-microliter solution of antibiotics was prepared and a filter paper was sodden and placed on assigned isolates. The plates were kept at 30 degree Celsius for 24 hours (Ali *et al.* (2016).

2.4. KOH test

In the Potassium hydroxide test, a 3% solution of KOH is prepared and a single drop of the solution is put on the slide. Then pick a colony of bacterial culture and mixed with the solution, and the slide was rotated for 15 seconds. Next, the colony is picked up by the toothpick that shows a thread-like structure. If the thread-like structure is shown it confirms that is a gram-negative bacterium (Ali *et al.* 2016).

2.5. Gram staining

The slide was washed with 95% ethanol then one drop of distilled water is added to the slide and a bacterial colony was picked and mixed with water. And the slide air-dried. Then, with the help of a dropper, crystal violet dye was added to the slide for 30 seconds. Rinse the slide with distilled water to remove the access dye. Then gram iodine was added to the slide for one minute. Then 95% ethanol was applied and rinsed the plate with distilled water. Then, safranine was applied and again rinsed the slide with distilled water. After this red color will show which confirms that the bacteria are gram-negative bacteria (Ali *et al*. 2016).

2.6. Pathogenicity tests

Carrot disk bioassay and potato disc confirm whether isolates cause a tumor or not.

2.7. Carrot and potato disc tests

In this test, the carrot was cut into small discs and washed with a 95% bleach solution for 2-3 minutes then rinsed with double distilled water. The filter paper was placed on the Petri plates where the carrot discs were placed. Then bacterial colony was picked and poured on each disc. These plates were kept in an incubator for 20 days to check the gall formation. The same method was repeated on the potato (Ali *et al.* 2016).

3. EXPERIMENTAL OUTCOMES

Plant science research fasces several problems by steady increase in the population globally, including demand for increase nutritional quality, grain yield, crop productivity. Climate change is also a big challenge today.

Through with many other inventions the discovery of *A. tumefacient* is used as an engineer in plant biotechnology. Genetic transformation of plants is relying on the *A. tumefacient*, a bacterial pathogen used for the transfer for our gene of interest into host plant cells. This bacterium contains the Ti plasmid (tumor inducing plasmid) which contain tumor causing genes, we can use this Ti plasmid as a vector for the transformation of our gene of interest. *A. tumefacient is* used as a carrier for the transformation of gene of interest into host plant cells. It is used as tool in genetic engineering for the production of genetically modified plants. Because it has natural ability to transfer their genetic material into host plant cells that's why it is more important in plant biotechnology. Genetic manipulation of *A. tumefacient* cause increases the transformation efficiency of genetic material into host plant cells.

Declarations

Source of Funding

This research work did not receive any grant from funding agencies in the public or not-for-profit sectors.

Competing Interests Statement

The authors declare no competing financial, professional, or personal interests.

Consent for publication

The authors declare that they consented to the publication of this research work.

Authors' Contributions

All authors equally contributed to research and paper drafting.

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